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Clinical Findings on Fibroblast Activation Protein in Patients with Gastric Cancer

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Human fibroblast activation protein (FAP) is a 97-kDa surface glycoprotein expressed in tumor-associated fibroblasts. In this study, we immunohistochemically examined FAP levels in surgically resected gastric carcinomas and explored their association with clinicopathological findings and prognosis. Sections of paraffin-embedded specimens were obtained from 100 patients with advanced gastric cancer between 1989 and 2001 at our institution, and they were stained with an anti-FAP antibody. Expression of FAP was detected in 64 patients (64%). Lymphatic vessel invasion was observed in 90% of FAP-positive patients ($P = 0.015$). Blood vessel invasion was observed in 98% of FAP-positive patients ($P < 0.001$). The disease-specific 5-year survival rate of in the 64 patients with FAP-positive tumors (22%) was significantly lower than in the 36 patients with FAP-negative tumors (34%, $P = 0.036$). This indicates that vessel invasion is connected with the expression of FAP and that a positive finding of FAP confer a worse prognosis in the patients with gastric cancer.

Key words: fibroblast activation protein; gastric cancer; immunohistochemistry; prognosis

Gastric cancer is one of the most malignant tumor types having a dismal prognosis. Lymph node metastasis, hematogenic metastasis, and peritoneal metastasis have all been reported to have a strong impact on prognosis for gastric cancer patients. Tumor cell invasion and subsequent distant spread via blood and lymph vessels are critical steps in tumor progression. In the process of tissue invasion and metastasis, cancer cells produce enzymes that degrade basement membranes and extracellular matrix (Liotta et al., 1991). During disease progression, interactions between cancer cells and mesenchyme-derived stromal cells, such as fibroblasts, myofibroblasts, endothelial cells, smooth muscle and hematopoietic cells occur. However, the relationship between cancer cells and mesenchyme-derived stromal cells has not been well described (Mueller and Fusenig, 2004; Huber et al., 2005; Joyce, 2005; Orimo et al., 2005; Kalluri and Zeisberg, 2006; Elinborg et al., 2008).

Human fibroblast activation protein (FAP) is a 97-kDa cell surface glycoprotein with gelatinase and depeptidyl peptidase activity (Pineiro-Sanchez et al., 1997; Park et al., 1999). This protein is expressed in stromal fibroblasts located in and around the tumor. The distribution of FAP is unique. FAP is not expressed by normal fibroblasts, but is expressed transiently in healing wound tissues (Ghersi et al., 2002), chronic inflammatory conditions such as cirrhosis (Levy et al., 2002) and some fetal mesenchymal tissues. Recently, FAP produced by stromal fibroblasts

Abbreviations: FAP, fibroblast activation protein; PBS, phosphate buffered saline

was reported to play an important role in tumor cell progression (Allinen et al., 2004; Narra et al., 2007). Cheng et al. (2002) reported that forced FAP overexpression by tumor cells in an animal model results in a significant enhancement of tumor growth.

In the present study, we investigated the clinical significance of stromal fibroblast FAP expression in gastric cancer by the immunohistochemical examination. We examined the relationship between FAP expression and clinicopathological findings in patients with gastric cancer. We also comment on the clinical importance of FAP expression in stromal fibroblasts.

Materials and Methods

Patients

The medical records of corresponding patients were retrospectively reviewed. The 100 primary gastric cancer patients were enrolled in this study. The stages of their tumors were III and IV. They underwent gastrectomy at our institution between 1989 and 2001. The age of patients ranged from 33 to 93 years (average 66.3 years); 72 were male and 28 were female. The clinicopathological findings were described according to the Japanese Classification of Gastric Cancer by our hospital pathologists (Japan Research Society for Gastric Cancer, 1995). All patients had undergone distal, proximal or total gastrectomy with dissection of level 1 and 2 regional lymph nodes (D2). Curative operations were performed in all patients. The follow-up periods for survivors ranged from 1 to 180 months (median, 39 months) after surgery. Informed consent was obtained from all subjects and/or their guardians.

Immunohistochemistry

A streptavidin-biotin kit (Histofine SAB-PO kit; Nichirei, Tokyo, Japan) was used for staining of formalin-fixed and paraffin-embedded specimens. Specimens were dewaxed in xylene, rehydrated in ethanol, treated with 0.3% hydrogen peroxide in methanol for 15 min, and then heated in an autoclave oven (120˚C) for 20 min. They were then washed with phosphate-buffered saline (PBS) and preblocked with 10% gout saline for 10 min. After washing with PBS, the samples were incubated overnight at 4˚C with an anti-FAP polyclonal anitibody (ab53066; Abcam, Tokyo) diluted at 1:50. Specimens were then washed in PBS and incubated with anti-rabbit immunoglobulin conjugated to biotin for 10 min, followed by incubation with a streptavidin peroxidase complex for a further 5 min. Following another wash with PBS, immunohistochemical labeling was visualized using freshly prepared diaminobenzidine tetrahydrochloride. Sections were counterstained with hematoxylin.

Scoring system

Stromal fibroblasts with FAP immunoreactivity were quantified by 2 independent observers who evaluated at least 1,000 fibroblasts in consecutive sections of neoplastic tissues. Stromal fibroblasts were classified into two categories based on FAP expression: i) negative staining (negative or equivocal staining or when less than 10% cells were detected) and ii) positive staining (when 10% or more cells were detected).

Statistical analysis

The chi-square test was used to compare the differences between the two groups. The significance of differences among means was determined by the Mann-Whitney U test. The overall and disease-free survival periods were estimated using the Kaplan-Meier method and compared using a two-sided log rank test. Cox's proportional hazards regression model was used to estimate the predictive power of FAP expression on clinical outcome. Two-sided tests were computed, and $P < 0.05$ was considered statistically significant.

Results

FAP expression and clinicopathological findings

FAP expression was detected in stromal fibroblasts around or within cancer tissue (Fig. 1). It was not detected in cancer cells or stromal cells located in normal gastric tissues in the resected specimens. FAP-positive stromal fibroblasts were detected in 64 patients (64%). The clinicopathological characteristics of the 64 patients with FAP-positive stromal fibroblasts were compared with those of the 36 patients who were FAP negative (Table 1). Lymphatic vessel invasion was observed in 90% of FAP-positive patients compared with 70% of FAP-negative patients (P = 0.015). Blood vessel invasion was observed in 98% of the former group compared with 19% of the latter group (P < 0.001). There was no significant difference with FAP expression level and histological type of gastric cancer.

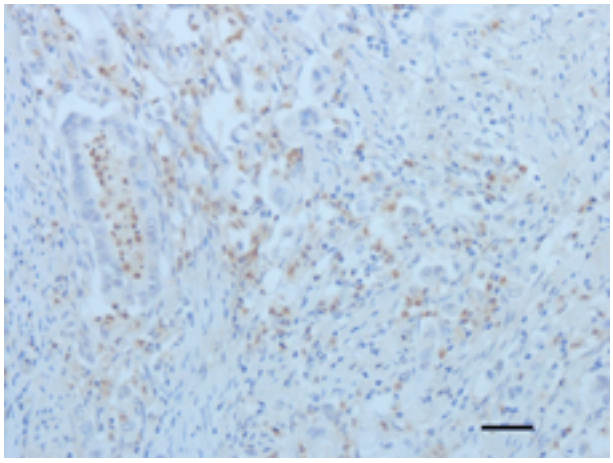

Fig. 1. Immunohistochemical staining pattern for FAP in a gastric carcinoma. Expression of FAP is detected in stromal fibroblasts. Bar = 50 μm.

Prognosis of patients

The overall 5-year survival rate among the 100 patients with stage III and IV gastric cancer was 29%. The disease-specific 5-year survival rate among the 64 patients with FAP-positive tumors (22%) was significantly lower than that of the 36 patients with FAP-negative tumors (34%, P = 0.036,

Table 1. Expression of FAP and clinicopathological parameters

		FAP expression		*P* value
		Positive	Negative	
Age (year)		67.2 ± 11.7	64.6 ± 9.8	0.26
Gender	Male	47	25	0.8169
	Female	17	11	
Tumor size (cm)		8.6 ± 3.7	7.6 ± 3.9	0.23
Cancer stroma relationship	Med, Int	31	17	0.0760
	Sci	31	20	
Histology*	Differentiated	24	12	0.8285
	Undifferentiated	40	24	
Lymph node metastasis	Absent	3	6	0.3536
	Present	61	30	
Lymphatic vessel invasion	Absent	6	11	0.0151
	Present	58	25	
Blood vessel invasion	Absent	1	29	< 0.0001
	Present	63	7	
Stage	III	43	29	0.172
	IV	21	7	

FAP, fibroblast activation protein; Int, intermediate type; Med, medullary type; Sci, scirrhous type.
* Differentiated, papillary or tubular adenocarcinoma; undifferenciated, poorly differenciated or undifferenciated adenocarcinoma or signet ring cell carcinoma.

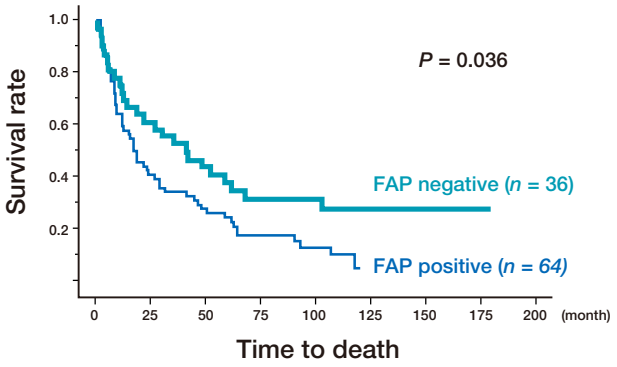

Fig. 2. Survival is estimated using the Kaplan-Meier methods, which produce the disease-specific survival curves of patients with stage III and IV gastric carcinoma according to the expression of FAP. FAP-positive cases have lower survival rates.

Fig. 2). Multivariate analysis was performed with a Cox proportional hazards model using the covariates of tumor histology, histological stage and expression of FAP (Table 2). FAP expression was not detected as a prognostic factor independent of the histological stage ($P = 0.072$).

Discussion

In the present study, we have immunohistochemically demonstrated the frequent occurrence of FAP expression in surgically resected gastric cancer. Our data showed that FAP expression is correlated with lymphatic and blood vessel invasion and caused a worse prognosis in patients with advanced gastric cancer. FAP expression has been described to be present predominantly in the tumor stroma of epithelial malignancies (Garin-Chese et al., 1990; Ariga et al., 2001), and its presence has been associated with increased microvessel density (Huang et al., 2004). FAP activity has also been suggested to participate in the growth of human colorectal tumor xenografts, because inhibition of FAP enzymatic activity in these tumors resulted in tumor growth attenuation (Cheng et al., 2002; Cheng et al., 2005). We have found strong relationship between FAP expression in tumor-associated fibroblast and invasion of cancer cells into microvessels of the gastric wall. These reports suggest that FAP-dependent pathways may play an important role in epithelial cancer invasion, tumor angiogenesis, and subsequent growth and metastasis.

We have found a statistically significant association between the expression of FAP and shortened survival time in patients. We have also found that the expression of FAP is an independent prognostic factor as well as the histological stage. It has been proposed that scirrhous gastric cancer has a worse prognosis, but in our study there was no siginificant difference in FAP expression between patients with scirrhous-type cancer and other types of cancer ($P = 0.076$). FAP pathways may predominate early in the course of smaller tumors to facilitate tumor invasion and tumor motility, which are required for metastases to occur, thus leading to the enhanced induction of FAP expression in early-stage disease. In advanced tumors, a relatively persistent elevation of FAP levels may signify a more aggressive tumor and a continued additive contribution of FAP-dependent pathways to tumor invasion and growth. Thus high FAP levels may confer a worse prognosis even in those with advanced disease (Leonard et al., 2007).

In conclusion, we found that stromal FAP levels are related to stage III and IV gastric can-

Table 2. Multivariate survival analysis by the Cox proportional hazards model

Variable	Hazard ratio	95% Confidence interval	*P* value
Tumor histology*			
Differentiated/ undifferentiated	0.861	0.542–1.368	0.527
Histological stage			
Stage III/IV	3.39	2.044–5.622	< 0.001
FAP expression			
Positive/negative	1.557	0.956–2.535	0.072

FAP, fibroblast activation protein.
* Differentiated, papillary or tubular adenocarcinoma; undifferenciated, poorly differenciated or undifferenciated adenocarcinoma or signet ring cell carcinoma.

cer, and that the expression of FAP is associated with decreased survival time. If successful, stroma-directed therapy may provide clinical benefits in a broad spectrum of indications, based on the consistent presence and remarkable abundance of stromal compartments in human carcinomas.

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